RADIATION ANALYSIS OF A LECANOID GENETIC SYSTEM[1]

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IN 1921, SCHRADER first reported the unusual cytological system in a mealy bug *Pseudococcus nipae* (Mask.). Further work by SCHRADER (1923a) and HUGHES-SCHRADER (1935) resulted in an exact characterization of a system of chromosome behavior which has been named "lecanoid." These and other studies are discussed in HUGHES-SCHRADER's (1948) comprehensive review of coccid cytology. One set of chromosomes undergoes facultative heterochromatization in male embryos and becomes excluded from the genetic continuum at spermatogenesis.

Although a striking departure from the more conventional forms of chromosome behavior, the lecanoid system is not a passing accident but has sustained quite considerable evolution. It occurs throughout a series of diverse families including the primitive mealy bugs (Pseudococcidae), the cochineal dye insects, the lac insects, the soft scales (Lecaniidae), the asterolecanoids, and the conchaspids as well as the most specialized coccids, the armored scale insects (Diaspididae) in which it occurs as a dependent system (HUGHES-SCHRADER 1948; BROWN 1959).

In the lecanoid system both males and females are diploid. One haploid set becomes heterochromatic at the blastula stage in the male embryos but otherwise the chromosomes of the two sexes are not distinguishable. The heterochromatic set divides synchronously with the euchromatic but forms a conspicuous chromocenter in the resting nuclei. Chromosome behavior is usually the same in soma and germ line; however, according to recent observations (BROWN, unpublished; NUR, unpublished), heterochromatization is frequently absent from certain polyploid nuclei and may be restricted to the germ line in a few species.

At spermatogenesis (Figure 1), the first mitosis is equational for both types of chromosomes while the second is segregational, the heterochromatic set moving away from the euchromatic on a monopolar or highly asymmetric spindle. Only the euchromatic derivative forms sperm; the heterochromatic entity forms a highly pycnotic residue which is maintained during much of spermiogenesis. Chromosome comportment in the female is normal during ontogeny and

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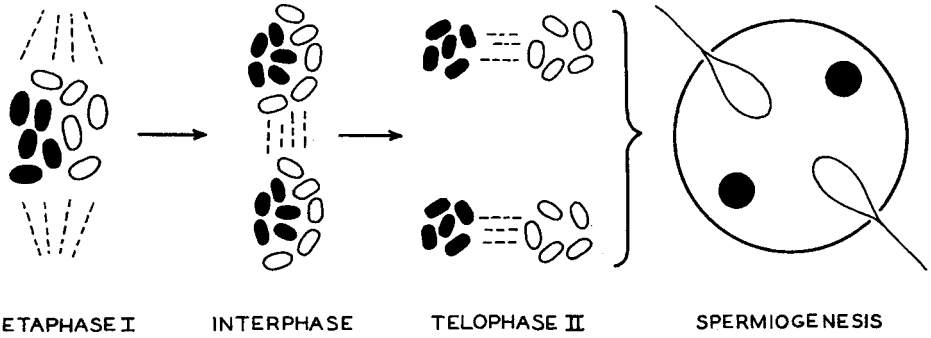

FIGURE 1.—Diagram of lecanoid spermatogenesis. The euchromatic and heterochromatic chromosomes divide equationally during the first division, and are segregated during the second. Spermatids contain all four products of the two divisions. Only the euchromatic derivatives form sperm; the densely pycnotic heterochromatic residues eventually disintegrate.

oogenesis. The presence of chiasmata in the bivalents may be taken to mean that recombination occurs by crossing over as well as by random assortment.

SCHRADER and HUGHES-SCHRADER (1931) believed the lecanoid system to be a stepping stone toward true haplodiploidy. The lecanoid male transmits only a single set of chromosomes and thus will breed as a haploid if this set were derived exclusively from either parent. SCHRADER and HUGHES-SCHRADER (1931) suggested that the heteropycnosis of one set of chromosomes indicates its genetic inertness; the male would therefore be a virtual haploid. HUGHES-SCHRADER (1948) further suggested that the heterochromatic set is of paternal origin. The present study was undertaken to test the validity of these two hypotheses by means of response to irradiation as suggested by P. W. WHITING's (1937, 1938) studies of the true haplodiploid system of Habrobracon.

MATERIALS AND METHODS

The laboratory stock culture of *Planococcus* (= *Pseudococcus*) *citri* (Risso) was obtained in August, 1957, from DR. D. C. LLOYD of the Commonwealth Institute of Biological Control, Fontana, California, and stock cultures were maintained by transferring eight to ten laying females each generation. The mealy bugs were grown on Irish potatoes, *Solanum tuberosum* L., wedged into glass jars with metal lids which were vented and screened with linen cloth. Since overcrowding may contribute to contamination, apparently by influencing the crawlers to explore rather than settle on the available potato, care was taken to prevent it. When any culture was terminated, it was autoclaved before opening. The laboratory was maintained at a mean temperature of 23°C.

Males and females of desired ages were obtained by withdrawing small numbers of females, usually four, at the start of oviposition and transferring them daily to fresh culture jars for a total duration of 9 to 11 days. Virgin females in the third instar were then later available from the daily-lay cultures before any of the males in the same jar had emerged from their cocoons as winged adults.

The males could either be collected for experimental crosses directly from their subculture jars or, since they do not feed after the end of the second instar, isolated in large numbers on cleansing tissue paper and maintained in corked glass vials. The transfers of individual insects were performed with slightly moistened camel hair brushes.

Except in the experiment designed to test the fertility of sons following paternal irradiation, all matings were made shortly after treatment and were initiated *en masse* on fresh potatoes to increase the likelihood of fertilization. Females were subsequently transferred to individual jars as soon as they began to lay their eggs. For irradiation males and females were placed in gelatine capsules. Controls were handled in the same way, including transportation to the radiation sites. X-irradiation was performed with a Phillips therapeutic X-ray unit and exposures were made with settings of 15 ma, 250 kvp, at a distance of about ten cm from the target and filtered with 0.5 mm aluminum. The Victoreen r-meter registered values of approximately 400r per minute. For the high-dosage series a cobalt^{60} chamber was used which delivered 3,333rep per minute at an opening of one inch. The radiation was performed at the Lawrence Radiation Laboratory through the courtesy of the Bio-organic Chemistry Group, University of California.

The progeny were counted as adults with the aid of a dissecting microscope. To avoid confusion, the individuals were either transferred to new potatoes for further crosses or destroyed during the counting process. Unhatched eggs were counted directly in the single ovisac left by the mother or if their number was large, the ovisac was transferred to a small sheet of filter paper and the embryos "floated out" on a larger area with a few drops of 95 percent ethyl alcohol.

About 3½ days following fertilization, gravid females contained a sufficient number of both male and female embryos for a cytological comparison to be made between them, i.e. those showing no signs of heterochromatization at late blastula and those at the same stage of development, but where heterochromatization of one set of chromosomes was just becoming apparent. Females of this age were stained and squashed in acetocarmine following fixation in BRADLEY's (1948) modification of Carnoy's fluid to which was added a very small amount of a saturated solution of ferric acetate in propionic acid. Embryos at the proper stage of development and with division figures could be selected without prejudice under low power and then analyzed for aberrations with an oil immersion objective; there were thus almost no unanalyzable embryos.

Life cycle of the mealy bug: Extensive embryonic development occurs prior to laying which commences about 4–6 days after mating and continues for nine to 11 days. The eggs hatch about nine days after laying and the freshly emerged crawlers wander around until they find a suitable place to feed. During the first and second instars, the males and females cannot be distinguished externally. Both are sedentary in habit and can be moved only with care not to break the very delicate feeding tube inserted into the plant. After the second instar the male stops feeding and undergoes metamorphosis inside a cocoon. Cracks and crevices are frequently chosen as sites for the pupation process. Spermatogenesis

is completed early in the third instar; spermatids develop synchronously and all sperm are mature when the male emerges as a winged adult 36–40 days after oviposition. He does not feed as adult, may mate with as many as 23 females (JAMES 1937) and dies within two to four days. He is extremely fragile, cannot be successfully anaesthetized, and must be handled individually and with care; he usually walks rather than flies, especially when freshly emerged.

The female can be mated soon after the last molt but will produce no offspring until full sexual maturity is attained. In her external morphology, she maintains the characteristics of the larval period and continues to feed until the end of oviposition. The sex ratio in JAMES' (1937) material was approximately 1:1; in our laboratory with a different strain and different cultural conditions, the ratio is approximately 0.67 males per female (NELSON-REES 1960). Fluctuations in the sex ratio and other reproductive characteristics of the female will be considered in the discussion.

RESULTS

Analysis of the genetic system of the mealy bug, *P. citri*, through its response to radiation consisted essentially of two parts, the influence on the progeny following treatment of either parent, and the transmission of induced effects. Both the genetic and cytological data will be presented before assessing the results in terms of the SCHRADERS' hypotheses. The data are in general quite consistent in their bearing on the hypotheses in question; however, the female mealy bug presents many complex problems in regard to variable sex ratio and variable and incomplete sexual dichronism of offspring. Though of secondary interest in regard to present purposes, the discrepancies stemming from such complexities require consideration. During the course of the present studies, a considerable number of radiation effects were discovered which merited detailed investigation, outside the scope of the present problem. Where it will help to clarify otherwise puzzling aspects of the present results, the progress to date in the subsequent studies will be summarized where pertinent in this section and the discussion.

X_1 response[1]

Maternal treatment: The average number of survivors per treated female is cited in Table 1 for two different experiments in which the age of the mothers differed. The survival curves, expressed in percentages of the control values of the same sex, are illustrated in Figure 2. The influence of maternal age on response to radiation has been the subject of extensive work (NELSON-REES 1960) which will be summarized in the discussion; the differences between the two experiments reported here are of the sort expected from the different maternal ages. In general, it may be concluded that maternal treatment results in marked lethality in both sexes of offspring with increasing dosage from 0 to 8,000r.

[1] In this report, the terms X_1 and X_2 will be used to indicate the first and second filial generations, respectively, in the experimental series.

TABLE 1

Average number of survivors per mother after maternal treatment

Dose	Series no. 1* n	Av. no. ♀♀	Av. no. ♂♂	5* n	Av. no. ♀♀	Av. no. ♂♂
0r	10	295.1	172.4	8	141.5	130.1
1,000r	21	45.7	59.9	11	123.4	134.0
2,000r	10	40.6	26.4	11	71.4	66.4
4,000r	7	4.1	1.4	10	19.2	12.1
6,000r	11	0.1	0.2	10	8.7	1.5
8,000r	10	0.2	0	10	1.8	0.1

* Females of age 33 days, series 1, and 42 days, series 5 when treated and mated.

Cytological examination following maternal treatments at 4,000 and 8,000r showed a marked increase in chromosomal aberrations in the embryos (Table 2, Figure 3). The normal diploid chromosome number for both sexes of *P. citri* is ten; in males, five chromosomes normally become and remain heterochromatic. There are no marked differences in chromosome size; the nucleolus organizer is near the middle of one chromosome which often appears disjunct at this region. Among the three control series, an unexpectedly high percentage of spontaneous alterations occurred in series B. The reason is unknown but was presumably not genetic since all series were drawn from the same stock culture. The types of aberrations are summarized in Table 3. Only those embryos were classed as

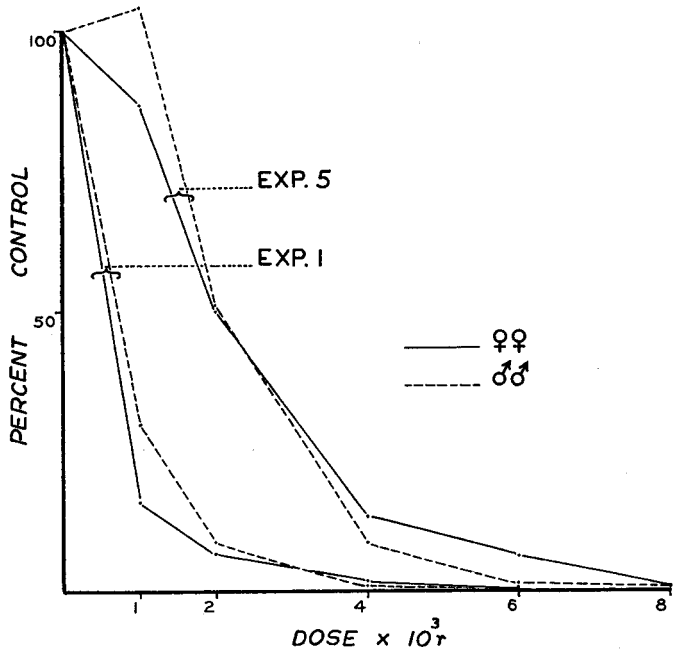

FIGURE 2.—Average percent survival to maturity of the X_1 following maternal treatment.

TABLE 2

Percentages of embryos with altered chromosome sets following maternal, paternal, and combined treatments

Treatment	Series	Age of mothers in days	No. of females	Male embryos				Female embryos		Young embryos	
				No. of embryos	Aberrations in H set	Aberrations in E set	Not distinguishable	No. of embryos	Aberrations present	No. of embryos	Aberrations present
Control											
	B	56–61	4	80	1.3	5.0	2.5	137	16.1	33	6.1
	C	45–46	9	100	0.0	4.0	0.0	26	3.8	30	0.0
	E	69	6	100	1.0	1.0	0.0	42	0.0	13	0.0
Maternal 4000r											
	B	56	1	37	0.0	32.4	10.8	29	37.9	22	50
	E	47	9	110	0.0	36.4	0.0	73	50.7	31	48.4
Maternal 8000r											
	C	43–46	19	114	1.7	37.7	1.8	42	71.4	15	93.3
	E	47–49	13	106	0.0	64.2	4.7	89	75.3	14	85.7
Paternal 8000r											
	D	55	3	52	90.4	0.0	0.0	28	92.9	11	90.9
	E	49	7	103	96.1	0.0	1.0	42	95.2	25	96.0
Combined maternal & paternal; each 8000r											
	E	47	4	37	24.3	0.0	. .	4	100	3	100
				(both H & E: 73.0)							

mosaics in which one or more division figures were normal and one or more showed chromosome aberrations; this class presumably resulted from postzygotic disturbances. As would be expected, relatively more mosaics of altered and normal cells were found in the control than in the experimental series since treatment would usually result in damaged gametes rather than postzygotic changes. In the control series (Table 3), the difference between the sexes in the type of abnormality was a function of the sampling; relatively more fragmentation occurred in series B, in which a disproportionately large number of female embryos were scored, than in series C and E in which more males were examined. Furthermore in series B the fragmentation occurred preponderantly in the female embryos.

A comparison of maternal treatments of 4,000 and 8,000r (Table 2) shows that doubling the dose brought about a substantial increase in chromosomal changes in young and female embryos. In regard to the affected male embryos, those of series E increased with increasing dosage but at 8,000r those of series C were about equal to that expected following a dose of 4,000r. It may be suggested again that maternal age was the responsible factor; the mothers of series C were somewhat younger than those of series B or E.

The types of aberration found after female treatment (Table 3) consisted largely of elimination of single chromosomes, and fragmentation. Another class,

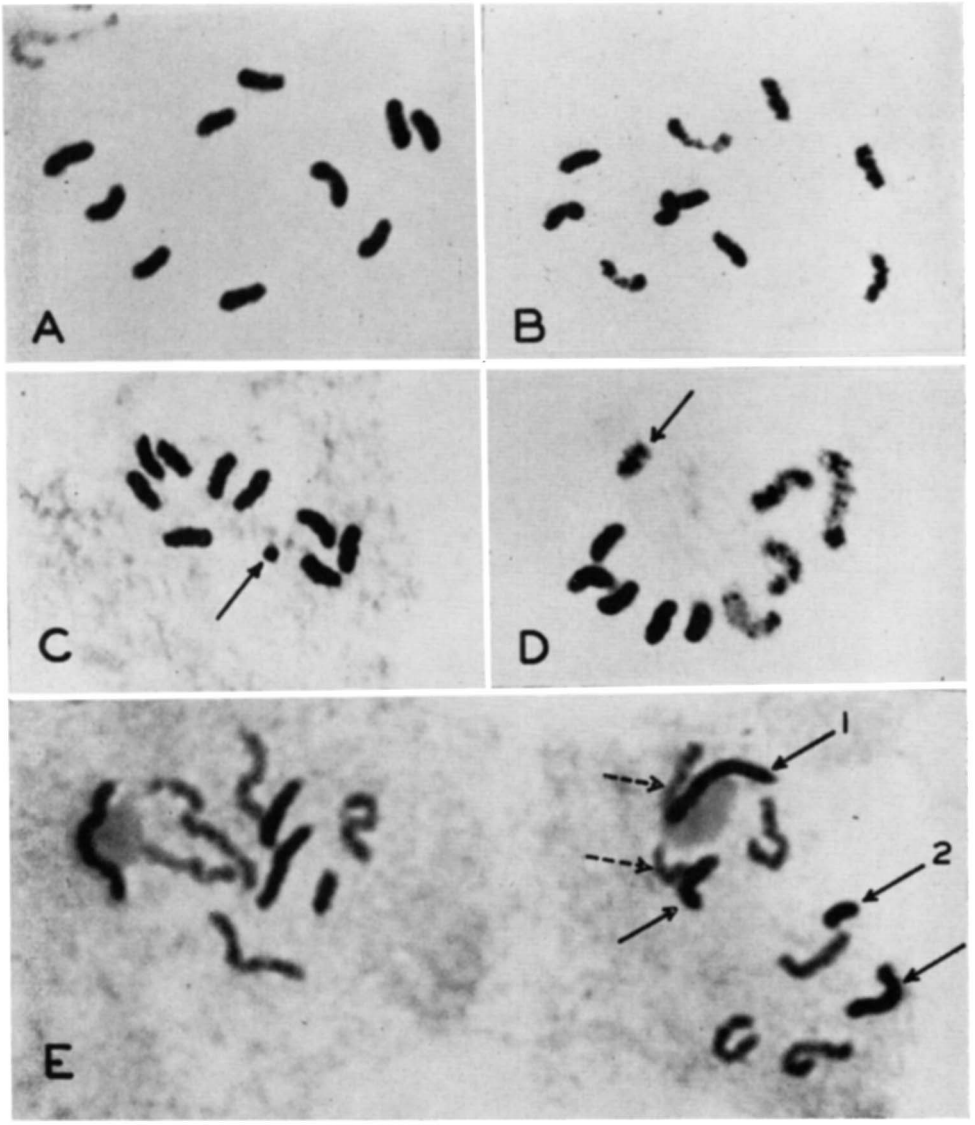

FIGURE 3.—Mealy bug chromosomes. A. Normal female set of ten chromosomes. B. Normal male set of ten chromosomes, five euchromatic, five heterochromatic, at late prophase. C. Nine chromosomes plus fragment in female embryo in which the euchromatic and heterochromatic sets cannot be differentiated. D. Male embryo; euchromatic set of four chromosomes and fragment (arrow). E. Male embryo, two adjacent cells; euchromatic set normal with typical subdivision of nucleolar chromosome (dashed arrows) on either side of submedian organizer; heterochromatic set (solid arrows) altered; of the four chromosomes, "1" is longer, "2" shorter than normal.

TABLE 3

Percentages of various chromosome aberrations in affected X_1 embryos cited in Table 2

		No. of embryos	9 or fewer chromosomes	9 or fewer chrom. & f.	10 chromosomes, size change*	10 chromosomes & f.	11 or more chromosomes
Control, non mosaic	♂♂	11	54.5	9.1	0.0	9.1	9.1
	♀♀ + young	26	15.4	11.5	7.7	7.7	7.7
Control, cont., mosaic†	♂♂	..	0.0	0.0	0.0	18.2	0.0
	♀♀ + young	..	0.0	0.0	0.0	50.0	0.0
Maternal treatment, non mosaic‡	♂♂	163	43.7	13.5	12.9	17.2	3.7
	♀♀ + young	203	28.1	23.2	16.2	20.2	4.4
Maternal treatment, cont., mosaic†	♂♂	..	1.2	0.6	0.0	6.7	0.6
	♀♀ + young	..	1.0	0.0	0.5	6.4	0.0
Paternal treatment	♂♂	97	7.9	40.3	25.2	20.1	6.5
	♀♀ + young	139	0.0	32.7	22.5	42.8	2.0

* Decrease or increase estimated at 1/2 to 1/3 chromosome length; smaller changes not identifiable; chromosomes less than 1/2 classed as fragments.
† Only those embryos in which some division figures showed a normal complement.
‡ Doses 4,000r and 8,000r combined.

in which there were nine or fewer chromosomes plus fragments, included a range from those which had undergone elimination and fragmentation to those in which the fragmentation itself accounted for the missing chromosome(s). Other abnormal complements were identified by the decrease in length of a third to a half of one of the chromosomes. Some mosaicism was found in which part of the embryo was normal and the aberrations in the abnormal sector may have been of spontaneous origin. In several cases, embryos were mosaics in which all sectors showed abnormal complements; these were classified according to the most nearly complete complement in the embryo; for example, an embryo with two sectors, one of nine chromosomes plus fragments and the other of nine chromosomes alone, would have been classified according to the former complement.

The observed abnormalities did not account for the total percentage of dominant lethals. After both doses in question, survival was less than ten percent. The embryos which appeared to have normal complements and to be developing normally at the stages examined must therefore have been carrying cytologically undetectable dominant lethals. Deficiencies up to a fourth of the length of a chromosome would not have been recognizable.

Of special interest was the almost complete absence of bridges during early embryogenesis. In the entire series examined, only three bridges were found; when they did occur, they were sufficiently striking to be readily detected. The class of young embryos included immediately postzygotic individuals, and these did not show abnormal arrangements of nuclei that might have been expected from chromosome bridging or other mechanical effects. It may therefore be concluded that postzygotic breakage-fusion-bridge cycles arising from radiation-induced breaks contributed little or nothing to dominant lethality following maternal treatment. Bridging at oogenesis was much more common and was probably the source of a high proportion of embryos lacking one or more chromo-protein takes place. Thus it has been assumed that thymine starvation causes an irreversible

somes; the types of structural alterations responsible for oogenic bridging are not known.

In spite of the complexities and inconsistencies in dosage response, the cytological data clearly demonstrated that only the euchromatic set of the male embryos was damaged following maternal radiation. The number of embryos with aberrations in the heterochromatic set was small and did not differ significantly from the control (Table 2). In some cases it was not possible to determine in which set the aberration had occurred. The two sets could be differentiated and analyzed for aberrations only during the middle to late prophase stages. If only prometaphase and later stages were available the sets could not be differentiated and such cases were recorded as questionable. The nature of small fragments could be determined only at midprophase before contraction reduced them to unanalyzable dots. By far the greater majority of fragments were, however, clearly and unmistakably identifiable as either heterochromatic or euchromatic.

Paternal treatment: Male treatments were made with both X-rays, over a dose range from 0 to 16,000r, and radiocobalt from 0 to 150,000rep (Table 4, Figure 4). The highest dose at which survivors were obtained was 120,000rep; after treatment at 150,000rep or higher the males either failed to mate or, if they did, to ejaculate since no sperm were found in the females with which they had been placed.

The survival curves differed strikingly from those obtained from maternal treatment. That of the daughters consisted of three parts, the low-dosage range (0–16,000r) in which the number declined with increasing dosage, the intermediate-dosage range (16,000r–30,000rep) in which fewer than ten percent survivors occurred, and the high-dosage range (60,000–120,000rep) in which the number was maintained at about 40 percent of the control value.

The curve for the sons consisted of two parts. In the first, equivalent to the low and intermediate sections for the daughters (0–30,000rep), the number of males recovered was usually greater than the control value. In the second, the high-

TABLE 4

Average number of survivors per mother after paternal treatment

X-ray series no.	n	4 ♀♀	♂♂	n	17 ♀♀	♂♂	n	18 ♀♀	♂♂
0r	5	212.8	163.8	11	245.2	167.2	9	248.6	182.2
2000r	2	114.0	129.0	10	248.0	199.3	11	164.4	164.1
4000r	1	58.0	203.0	10	126.2	190.0	10	180.3	191.7
8000r	3	41.0	188.0	12	47.7	198.4	10	101.2	239.8
16000r	3	3.0	275.0	12	7.1	145.7	10	11.9	273.5
Radiocobalt series no.		I			III			VI	
0rep	10	225.7	75.8	10	279.1	180.6	11	354.1	201.6
15000rep	8	14.2	95.0	11	7.9	181.3	11	70.2	281.6
30000rep	10	24.2	130.9	10	6.2	126.3	11	6.1	254.9
60000rep	10	144.8	3.8	11	89.5	2.9	11	125.2	35.4
90000rep	..	....	....	9	84.8	0.2	11	126.4	0.7
120000rep	9	118.0	1.3	3	114.0	0	6	112.7	0

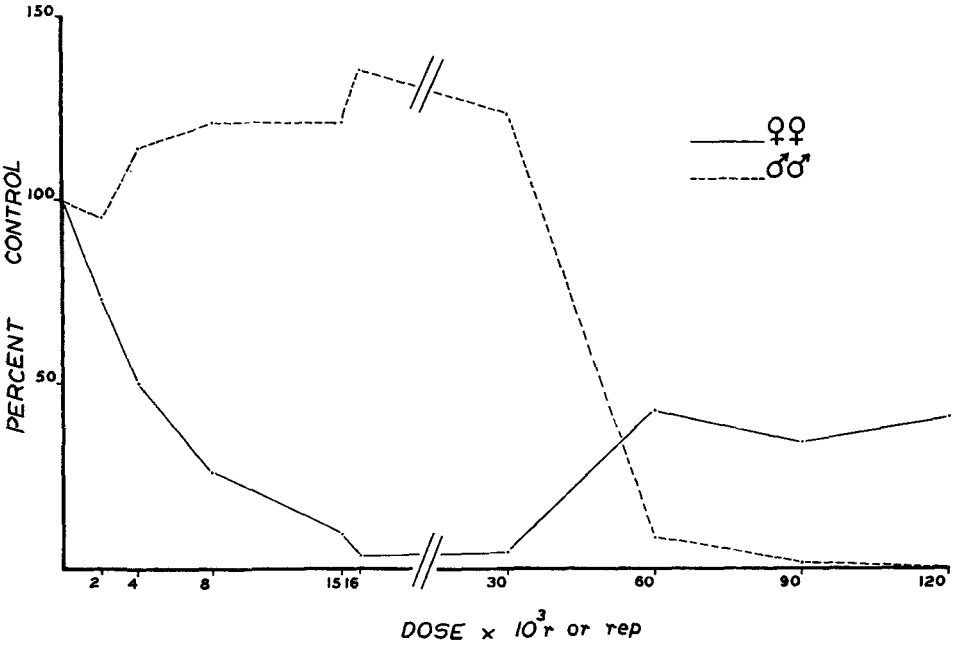

FIGURE 4.—Average percent survival to maturity of the X_1 following paternal treatment.

dosage range, the number of males was reduced abruptly to less than ten percent at 60,000rep and very few were obtained after treatments at 90,000 and 120,000rep.

Cytological examination of X_1 embryos after male treatment at 8,000r showed that over 90 percent had affected chromosome sets (Table 2, Figure 3). At the same dose, treatment of the fathers thus produced considerably more damage than treatments of the mother for all but the young embryos; however because of the complexities of assessing maternal treatment, this comparison is undoubtedly not completely fair. The paternal treatments yielded much more uniform results between the two experimental series and among the three classes of embryos than did the maternal.

The type of aberrations most commonly occurring after paternal treatment also differed. In relatively few embryos was a chromosome simply absent, and loss of a whole chromosome was usually accompanied by fragmentation (Table 3). Proportionately over twice as many female and young embryos as male carried ten approximately normal chromosomes plus one or more fragments. A large part of this discrepancy is no doubt attributable to the fact that the chromosomes studied in the two classes of embryos differed. In the male embryos, the aberrations occurred almost exclusively in the relatively more condensed heterochromatic set while in the female and young embryos all chromosomes are euchromatic; the residual section remaining after fragmentation would be more apt to be classified as a "fragment" rather than a "chromosome" if it occurred in a heterochromatic than a euchromatic set.

After paternal treatment of 8,000r, about 25 percent of the daughters survived yet only about five percent (average for series D and E) did not show some type of chromosomal change. All the observed changes were therefore not accompanied by dominant lethal effects.

The failure of appearance of mosaics of normal and aberrant complements after paternal treatment was probably a statistical effect. Irradiation resulted in about 95 percent aberrations, for the combined series D and E, for each of the three classes of embryos. A generous estimate of three percent of spontaneous aberration yielding mosaicism leads to an expected frequency of less than one case among the five percent which did not already show aberration.

Cytological studies of X_1 embryos and survivors are of special interest and are being continued as separate problems by CHANDRA and by NELSON-REES. Observations pertinent to the present study will be briefly summarized at this juncture. The few males surviving after high-dosage treatment clearly demonstrated the results of chromosome breakage in the heterochromatic sets; in most of the examples in which spermatogenesis was studied, the heterochromatic set consisted of a single very long chromosome, one or two normal chromosomes and several fragments (NELSON-REES unpublished). After high dosage treatment, one chromosome set appeared much like confetti in division figures in the X_1 embryos (CHANDRA unpublished). At anaphase the tiny fragments showed chromatin bridging which was difficult to analyze further but resulted in abnormal division figures and restitution nuclei. Of considerable interest was the observation that serious imbalance of the euchromatic and heterochromatic sets in the male embryos led to endomitosis and the formation of grossly polyploid embryos prior to death. In both the low and the high dosage series, pycnosis and other symptoms of necrosis were not apparent prior to gastrulation. CHANDRA (unpublished) further studied the cytology of the numerous females appearing after high-dosage treatment. Evidence of chromosome breakage was almost completely lacking. The females were usually triploid, but were occasionally diploid or diploid-triploid mosaics. The triploid females probably arose when the united polar bodies, which normally enter into the formation of the mycetocytes (SCHRADER 1923b), took over embryogenesis. The diploid females and diploid sectors of mosaics arose either from the second polar body or from zygotic derivatives in which the bridging of the damaged set had prevented its separation in mitosis and permitted the undamaged set to form independent daughter nuclei.

Cytological studies have not yet been made for the dose range of 15,000r to 60,000rep. Whether or not the formation of bridges is of gradual or abrupt onset, and the amount of damage to the zygotic derivatives required to permit triploid embryogenesis are at present open questions.

In conclusion, the results of paternal treatment demonstrate that there was little or no dominant lethality induced in male embryos in the dose range in which there were no mechanical disturbances in embryogenesis while the number of daughters decreased markedly over the same range. At the higher dose range, dominant lethality was induced at a high rate in the males while abundant chromatin bridging appeared in the embryos. As judged by the absence of aberra-

tions, the chromosomes of the females appearing after high-dosages were derived exclusively from their mothers. The damage to the male embryos stemming from paternal treatments was found almost exclusively in the heterochromatic set; similar results were obtained by CHANDRA (unpublished) after high-dosage treatments. The results of paternal treatment differed from those of maternal treatment by being in general much more consistent. In addition, after maternal treatment the percentage of dominant lethals was considerably higher than the percentage of embryos with detectable chromosome changes while the reverse was true following paternal treatment. Part of this difference is undoubtedly attributable to the completion of meiosis after radiation of the female.

X_2 and backcross progenies

Paternal treatment: The X_1 offspring obtained after various doses from 0 to 16,000r were allowed to cross *inter se* in the culture jars and the females were transferred before oviposition to individual cultures. X_1 males and females were also crossed individually to mates drawn from the stock cultures. The results of the three different sorts of crosses are given in Table 5 and the survival curves are pictured in Figure 5 as unweighted averages for the two experimental series. The differences between the two experimental series were probably attributable to cultural differences, such as the quality of potatoes, between the two tests as well as to the handling of the parental generation.

The X_1 *inter se* and the backcross of X_1 females to stock culture males gave essentially similar results. In both cases there was a steady decrease in the number of offspring of both sexes with increasing dose administered to the P_1 males. The percentage of survivors as calculated for each sex independently from its own control value was consistently lower for the males in both the X_1 *inter se* and the backcrossed X_1 females.

Cytological studies of the X_1 females surviving to maturity have been made

TABLE 5

Offspring of the X_1 obtained from paternal treatment

Dose	Exp. no.*	X_1 *inter se* n	X_1 *inter se* Av. no. ♀♀	X_1 *inter se* Av. no. ♂♂	X_1 ♀♀ × stock ♂♂ n	X_1 ♀♀ × stock ♂♂ Av. no. ♀♀	X_1 ♀♀ × stock ♂♂ Av. no. ♂♂	Stock ♀♀ × X_1 ♂♂ n	Stock ♀♀ × X_1 ♂♂ Av. no. ♀♀	Stock ♀♀ × X_1 ♂♂ Av. no. ♂♂
0r	6	9	361.2	283.3	..	....	....	..	....	....
	18	8	254.2	168.9	..	....	....	..	....	....
2,000r	6	10	172.9	80.3	6	187.5	78.7	5	346.6	227.2
	18	9	189.6	69.8	9	204.3	153.3	4	276.5	192.0
4,000	6	11	86.4	16.9	5	60.8	13.0	8	251.5	166.5
	18	6	42.6	16.7	10	148.6	31.5	10	250.3	173.4
6,000r	6	15	58.9	8.6	8	60.4	14.5	7	266.3	151.3
8,000r	18	8	29.6	7.8	9	19.0	3.6	8	216.0	214.4
16,000r	6	13	6.2	2.6	7	17.4	2.0	5	358.0	207.2
	18	8	2.1	0.1	5	2.4	0.6	7	129.1	99.1

* In the P_1, males were slightly older when irradiated in exp. 18 than in exp. 6; treated males were mated to 29–34 day old females in exp. 6, to 44 day old in exp. 18.

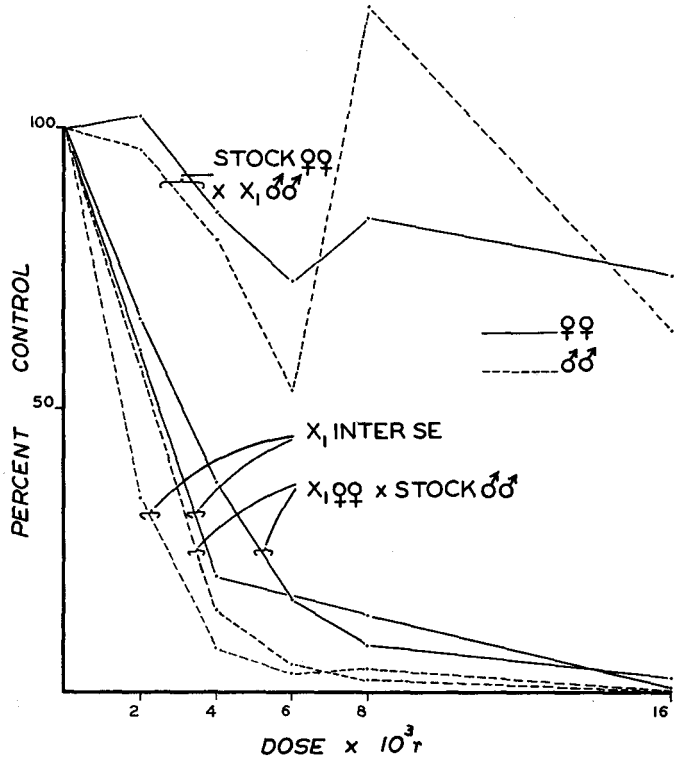

FIGURE 5.—Average percent survival to maturity of the X_2 and backcross progenies following original paternal treatment.

only to a limited extent; some but not all were seen to have altered chromosomes such as fragments, and others probably carried translocations which would not have been detectable unless grossly unequal. The partial sterility of the X_1 female has been assumed to be a consequence of the formation of duplication-deficiency gametes at oogenesis. Without prejudice as to the nature of the chromosomal changes involved, this type of dominant lethality may be referred to as meiotic-product lethality.

The X_1 males contributed nothing detectable to the results of the X_1 *inter se* since progenies similar to the X_2's were obtained when the X_1 females were backcrossed to the presumably normal stock culture males. However, when X_1 males were crossed with stock females, the total number of offspring decreased about 30 percent over the tested dose range (Figure 5). Statistical tests suggested by DRS. HENRY SCHEFFÉ and EVERETT R. DEMPSTER showed that the differences from control value were barely significant. The number of eggs which failed to hatch was not noticeably greater for this backcross than for the control series although a considerable increase in the number of unhatched eggs was invariably associated with both directly induced dominant lethality and that appearing in the X_2 or backcross progenies as meiotic-product dominant lethality. It seemed

likely, therefore, that if the X_1 males differed from the controls, they did so not in their transmission of lethals but in reduced vigor expressed as failure to fertilize effectively. These experimental series with the X_1 males gave the only results which seemed to contradict one of the hypotheses being tested. On the other hand, the difference from control may have stemmed simply from the very marked fluctuations in total productivity of the female mealy bug. The situation seemed, therefore, to merit further analysis.

A mealy bug female may mate either simultaneously (NELSON-REES 1959) or successively with more than one male. By giving the female more than one mate, compensation could be made for any lack of vigor shown by the individual males. An experiment was set up in which single females were given one, three, or six males for both the control series and the experimental. The results are summarized in Table 6. Females seemed to fail more often to produce any offspring when provided with only one male, but there were no noteworthy differences in this regard between the control and experimental series. Although there was here probably no cause and effect relationship, productivity declined in the control series with increase in the number of males but in the experimental series it varied apparently at random. The percentages for the experimental females were calculated from the productivity of the control female mated to the same number of males and for the two sexes independently. The fact that the percentages in the experimental series were usually over a hundred was probably attributable simply to the already noted decline in productivity of the control females with increase in number of males. In this experiment care had been taken to control more precisely the age of the mother as other work in our laboratory had meanwhile shown that a few days difference in the age of a sexually mature female could materially influence her total productivity (NELSON-REES 1960). As in the first experiment, there was no increase in the number of unhatched eggs in the experimental series. Within the limits of the second experiment, the males obtained after paternal treatment were as effective in mating

TABLE 6

Offspring of X_1 males (from paternal treatment) mated in different numbers to to individual stock females

Dose*	No. of males†	No. females Laying	No. females Not laying	Av. no. offspring‡ ♀♀	Av. no. offspring‡ ♂♂	Percent controls§ ♀♀	Percent controls§ ♂♂
0r	1	21	4	175.1	124.1	....	....
	3	23	0	167.6	106.4	....	....
	6	23	0	144.2	104.3	....	....
8,000r	1	21	1	163.7	138.3	93.5	111.5
	3	19	1	183.8	132.0	109.7	124.0
	6	16	1	172.9	137.5	119.9	131.8
16,000r	1	19	4	177.3	130.5	101.2	105.2
	3	24	1	140.5	112.8	83.9	106.0
	6	22	1	179.7	130.8	124.6	125.4

* To the P_1 male.
† Number of males placed with the individual stock females.
‡ Of those laying.
§ For the comparable 0r cell: sex and no. of males.

as those taken from stock cultures. The converse experiment, of mating individual males to varying numbers of females, was not performed.

The few X_1 males appearing after high dosage treatments (90,000rep) presented quite another picture. On mating, all have proved to be sterile; some were found on dissection to have defective testes but in others, the only detectable developmental abnormalities were some malformed spermatids (NELSON-REES unpublished). According to the cytological studies of CHANDRA (unpublished) described above, the high-dosage males must have survived an early embryogeny which was highly disturbed. The absence or failure of normal development of the testes may perhaps have been a reflection of greater derangement in the first cells of the germ line than elsewhre in the embryo. The converse condition, viable cells in the germ line but not in the soma, would not permit survival. A further possible cause of the observed sterility will be considered in the discussion.

Maternal treatment: Only a limited number of crosses have been made with X_1 individuals obtained following the more severe maternal treatments (Table 7). When X_1 females were tested, the number of offspring of both sexes declined with increase in the original dose and the males were considerably more affected than the females. The results were in general similar to those obtained with X_1 females following paternal treatment but considerably less severe at the same doses. Only three X_1 males were tested, two after 4,000r and one after 8,000r; on crossing to stock culture females, each gave an essentially normal progeny. Although the number of sons from male no. 1 was low, there was no corresponding increase in unhatched eggs as would be expected if lethal factors were the reason. Several daughters of each X_1 male were next tested and these all yielded normal progenies, again with no or few unhatched eggs.

DISCUSSION

Sex determination: The sex ratio of the total offspring of a female mealy bug

TABLE 7

Offspring of X_1 males and females obtained from maternal treatment

a. Average number of offspring of X_1 females

Dose	n*	Av. no. ♀♀	Av. no. ♂♂	Percent control ♀♀	Percent control ♂♂
0r	8	254.2	168.9	. . .	. . .
4000r	10	187.9	100.2	73.9	59.3
6000r	10	179.0	67.0	70.4	39.7
8000r	6	159.7	39.5	62.8	23.4

b. Offspring of three X_1 males and of their daughters

	X_1 male no.†	No. offspring		n‡	Av. no. ♀♀	Av. no. ♂♂
4000r	1	208	47	4	189.0	122.5
	2	243	212	5	269.6	167.0
8000r	3	372	123	7	268.7	193.6

* Mated with stock males.
† Mated with stock females.
‡ Daughters of X_1 males; mated with stock males.

may vary widely from mother to mother and is also markedly influenced by maternal age at mating and by environmental conditions (JAMES 1937, 1938). HUGHES-SCHRADER (1948) suggested, therefore, that sex determination is under weak genetic control, easily modified by other factors. No sex chromosomes have been identified in any of the lecanoid examples studied and, until the onset of heterochromatization in those embryos destined to be male, all normal embryos have identical chromosome complements.

NELSON-REES (1960) has confirmed JAMES' results and has also found, from a study of the daily-laying series, that the mealy bug exhibits an incomplete sexual dichronism which varies with the age of the mother. For example, females mated at 46 days of age produced a preponderance of male offspring during the first few days of oviposition while those mated at 56 days yielded mostly females during the same period. By choosing mothers of appropriate age, almost any type of brood pattern could be produced. This variation in sexual dichronism with age was independent of the influence of maternal age on the sex ratio of all her offspring since the increase in proportion of males did not begin until the mother was about 60 days old. Total productivity was also influenced by maternal age, rising to a peak some ten days after the last molt.

In NELSON-REES' radiation experiments, sex ratios among the offspring as well as total survival were markedly influenced by the age of the mother at the time of treatment and mating. These results were explained on the assumption that the oocytes destined to yield male or female embryos were differentially sensitive to radiation because they were, as indicated by the sexual dichronism, at different stages of maturation when treated. Similarly the varying effect of treatment on total productivity was a reflection of varying proportions of oocytes at different stages of development. The sort of changes in the sensitivity of oocytes presumed for the mealy bug have been demonstrated by ANNA R. WHITING (1945a,b) in Habrobracon and in Sciara by BOSEMAN and METZ (1949). Although the experiments of NELSON-REES have not provided an answer to the problem of sex determination in the mealy bug, they have revealed unsuspected intricacies in the process which must be taken into account in any experimental work.

The SCHRADERS' *hypotheses:* With the complexities described above in mind, we may now consider the bearing of the present results on the two hypotheses of the SCHRADERS. According to that which we shall refer to as hypothesis I, the heterochromatic set is of paternal origin. That the male mealy bug breeds as a haploid, transmitting only the euchromatic set, has been so well established by numerous cytological observations that it should be considered a fact; however, the bearing of certain experimental results on the conclusions from the cytology will be considered below. According to hypothesis II, the heterochromatic set is genetically inert and the male mealy bug a virtual haploid. With the exception of the requirement of fertilization in the mealy bug, the genetic system expected is thus exactly that of a typical haplodiploid insect in which the haploid males arise from unfertilized eggs. This statement should not be taken to imply that

secondary, residual, or special genetic or physiological activity is or is not carried on by the heterochromatic set.

Genetic factors in a haplodiploid organism are expected in general to be sex linked in the same fashion as X chromosome markers in an XX-XY mechanism. The simplest genetic test for the existence of such a system is that of the reciprocal cross but we have had available in the mealy bug neither genetic nor chromosomal markers.

The cytological studies after maternal and paternal treatments demonstrated quite convincingly that the heterochromatic set is of paternal origin and hypothesis I of the SCHRADERS is thus confirmed (Table 8a,c). The results after maternal treatment also indicated that radiation did not itself detectably induce heterochromatization because the effects of treatment were limited to the euchromatic set.

The demonstration of the genetic inertness of the heterochromatic set (hypothesis II) depended primarily on the failure of paternal treatment to induce dominant lethality in male offspring at doses severe enough to kill off more than 90 percent of the daughters (Figure 6A,B). Over 90 percent of the embryos of both sexes had altered chromosomes after paternal treatment at 8,000r. The chromosomes of embryos of both sexes sustained approximately the same amount of damage but in the male embryo the damage was restricted to the heterochromatic set. It may therefore be concluded that dominant lethality cannot be induced by uncomplicated damage to the heterochromatic set (Table 8b). The lethality at higher doses may be explained by the induced chromatin bridging and the consequent disturbances in embryogenesis. If bridging which resulted

TABLE 8

Summary of bearing of results on the SCHRADERS' *hypotheses*

	Hypothesis	
	I (Heterochromatic set paternal)	II (Heterochromatic set inert)
Paternal treatment—X_1		
a. Induced aberrations in heterochromatic set in male embryos	confirm	....
b. No dominant lethality in sons at lower doses (0r–30,000rep)	....	confirm
Maternal treatment—X_1		
c. Induced aberrations in euchromatic set in male embryos	confirm	....
d. Lethality induced in two sexes varying with maternal age (recessive lethal component not identifiable)	....	neutral
Paternal treatment—backcross		
e. X_1 males about as vigorous and fertile as stock males	....	1st exp. contradict 2nd exp. confirm
Paternal or maternal treatment—X_2 and backcross		
f. X_1 daughters yield proportionately fewer sons (recessive lethal component)	....	conform

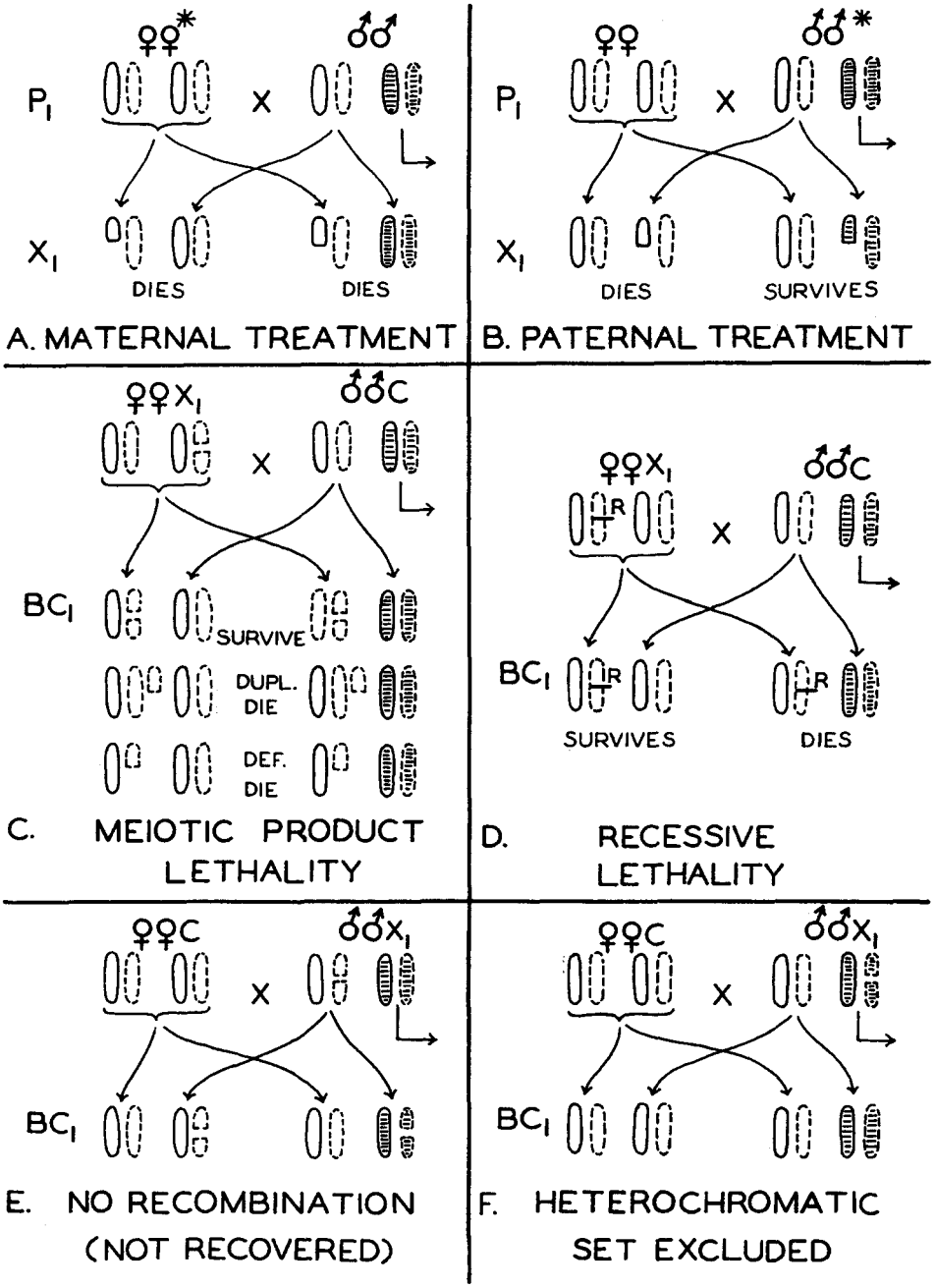

FIGURE 6.—The results expected from various crosses according to the hypotheses of SCHRADER and HUGHES-SCHRADER that the heterochromatic set (shaded chromosomes) is of paternal origin and is genetically inert. (See FIGURE 1 for diagram of lecanoid spermatogenic sequence). (C, stock culture).

in lethality were a regular sequel to chromosome breakage, dominant lethality in the X_1 males would have been expected to increase immediately and proportionately with paternal dosage regardless of the inertness of the heterochromatic set.

Further support for the validity of hypothesis II comes from the data on the progenies of X_1 females obtained from either paternal or maternal treatment. In these the proportion of males was consistently reduced to a greater extent than that of the females. In addition to the meiotic-product dominant lethals which kill both males and females (Figure 6C), the males would be expected to be killed also by recessive lethals if the heterochromatic set were inert (Figure 6D). Differential sensitivity of the oocytes was presumed to be responsible for much of the fluctuation in sex ratio among the progenies following upon maternal treatment (Table 8d); this factor would not be important in the reproduction of the X_1 females which were untreated. However, two other possibilities must be taken into consideration. The damage to the chromosomes carried by the X_1 females may have grossly influenced sex determination in favor of daughters in a manner analogous to the modifications in sex ratio induced by maternal aging and environmental factors. Secondly, the meiotic process itself may vary with the age of the mother; less regular pairing and assortment would thus have yielded a larger proportion of unbalanced gametes in those oocytes in the X_1 females destined to yield sons. Although both of these latter alternatives seem unlikely, neither can be ruled out at present; the data from the X_2 progenies, and those from backcrosses to stock culture males of X_1 females, should be regarded as conforming to the expectations of hypothesis II rather than directly confirming it (Table 8f).

The data from the first experimental crosses with X_1 males derived from paternal treatment indicated that these may have been hindered in effective fertilization. These results implied that damage to the heterochromatic set had a deleterious effect on the males and thus contradicted hypothesis II. Within the limits of a second, more extensive test, no significant difference between the

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A. Dominant lethals induced by maternal treatment(*) would be equally effective against sons and daughters.

B. Dominant lethals induced by paternal treatment(*) would kill only daughters; in the sons, the damaged chromosomes would be heterochromatic, inert and, without secondary complications, lead to no lethality.

C. A broken chromosome, otherwise not lethal, would be expected to result in some duplication-deficient gametes at oogenesis and lead to dominant lethality in both sexes. (See also E).

D. A recessive lethal (R) would be expected to kill the 50 percent of sons receiving it because the corresponding locus in the heterochromatic set is inert.

E. Parts of a broken chromosome will not be subject to recombination during spermatogenesis; thus no meiotic-product lethality will be expected. Altered chromosomes transmissible through the male have not yet been observed directly nor recovered, presumably because of associated recessive lethals. (See also F).

F. Damaged paternal derivatives will not be transmitted by sons; normal segregation at spermatogenesis of badly damaged heterochromatic sets (90,000rep) observed by NELSON-REES (unpublished).

control and experimental males was detected. The second experiment thus offers further confirmation of hypothesis II (Table 8e). In his original work with the mealy bug, SCHRADER (1921) believed that the Y chromosome(s) of the male was represented by the heterochromatic set; according to subsequent work (see HUGHES-SCHRADER 1948) the heterochromatic set does not form sperm and is therefore not transmitted similarly to a Y chromosome. SCHRADER's first interpretation should be kept in mind however in regard to possible genetic activity. The heterochromatic set or certain regions in it might thus have a function similar to the Y chromosome of *Drosophila melanogaster* which is necessary for the fertility but not the viability of the male (STERN 1929). Our results do not completely rule out the existence of such factors; they do indicate that there can be no more than a few active regions or the segmental losses revealed by the cytology of the low dosage X_1 male embryos (paternal treatment) would frequently lead to sterility. Part of the sterility of X_1 males following high-dosage paternal treatment may possibly be attributable to damage to fertility factors (NELSON-REES unpublished), as well as to the developmental derangements already suggested.

Confirmation of the numerous cytological studies of lecanoid spermatogenesis comes from the failure of X_1 males to yield meiotic-product lethals after either maternal or paternal treatment in which regard they differed strikingly from their sisters. The first spermatogenic division is equational for all chromosomes while the second segregates the euchromatic from the heterochromatic set; there would thus be no opportunity for recombination to produce the duplication-deficiency gametes presumed responsible for the meiotic-product lethality. The expectations from cytology would here remain the same whether the heterochromatic set were genetically inert or not and whether of paternal or maternal origin (but not if of mixed origin).

In studies of spermatogenesis in X_1 males following high-dosage treatments, NELSON-REES (unpublished) has observed the segregation of the damaged heterochromatic set, including fragments, from the euchromatic. Although insufficient yet to prove that all fragments, particularly the very small, will always behave regularly, these observations do confirm the expectations from the normal cytology that no recombination occurs between the two sets (Figure 6F).

Since the heterochromatic set is genetically inert, it is perhaps not surprising that we have not yet succeeded in finding cytologically demonstrable rearrangements transmissible through the male (Figure 6E). The three X_1 males from higher-dosage maternal treatment (Table 7) failed to transmit to their daughters the basis for meiotic-product lethality. Other, more extensive attempts also failed when X_1 females, following paternal treatment, were selected on the basis of reduced progenies. It seems likely, therefore, that many of the chromosomal changes were accompanied by recessive lethals and recovery of those viable in the male will require further trials with more stringent screening methods.

The hyperabundance of X_1 males: The increase in number of the X_1 males which appeared after paternal treatments from 4,000r to 30,000rep has been consistent enough to be regarded as more than a reflection of the vagaries in total productivity of the female mealy bug. The mealy bug embryo is at a fairly late

stage of development, with legs, antennae, and mouth parts quite well formed, at the time the egg is laid. Following treatment, embryos may die at any stage from gastrula onwards. On the assumption that the embryo continues to draw nutrients from its mother during most of its ovarian development, its death prior to oviposition would allow the mother to divert materials to the production of additional eggs and embryos; thus the total number of initial zygotes would be increased over that of the controls. If no lethality were induced in the sons, their total number should be increased over that of the controls while a higher percentage of females would survive than would otherwise occur.

Effectiveness of paternal and maternal treatments: Although it was outside the scope of the present problem to determine the relative sensitivity to irradiation of oocytes and sperm, a few of the striking contrasts between the two types of treatment will be briefly noted. At similar doses up to 8,000r, maternal treatments resulted in a much more drastic decrease in number of daughters. The effects on the sons cannot, of course, be compared. Cytological examination indicated considerably more apparent derangement in the chromosomes of X_1 embryos after paternal than after maternal treatment. Thus the surviving daughters would presumably have carried fewer chromosomal rearrangements after maternal than after paternal treatment. This difference may have been reflected in the tests of the X_1 daughters which yielded very much more reduced progenies after original paternal than after original maternal treatments at comparable doses. At any rate, the ratio of directly induced to delayed dominant lethality was obviously much higher after maternal than paternal treatment. It seems likely that part of this difference may be attributed to the screening effect of oogenesis which was completed after irradiation. Frequent elimination of smaller fragments during oogenesis would yield apparently normal chromosome complements which in reality were sufficiently deficient to be immediately lethal. The surviving females would therefore have fewer altered complements capable of yielding meiotic-product lethals.

Delayed dominant lethality and the holokinetic chromosome: In Drosophila, translocations will lead to the formation of duplication-deficient gametes; these in turn will result in the formation of inviable zygotes unless combined with a gamete carrying the complementary duplication and deficiency (MULLER and SETTLES 1927). Thus an X-ray induced translocation, though in no way lethal in itself, will yield dominant lethality in the X_2 generation. This type of delayed dominant lethality, which we have referred to as meiotic-product lethality, should be enhanced proportionately over that directly induced in organisms with holokinetic chromosomes. In a typical chromosome, a break in a chromosome arm will lead directly to a deficiency because the acentric portion will not be maintained, and the deficiency will often be sufficiently great to cause immediate dominant lethality. In a holokinetic chromosome, a similar break will lead to the formation of two fragments both of which may be maintained; there will thus be no deficiency and no immediate dominant lethality. Pairing behavior and assortment of the fragments at subsequent meioses is expected to be irregular, and at that time to lead to the formation of duplication-deficiency gametes.

With either type of chromosome, the initiation of a breakage-fusion-bridge cycle (McCLINTOCK 1938) might be expected to aggravate genetic inbalance. Bridges during cleavage have been observed after parental treatment in Habrobracon (ANNA R. WHITING 1945a,b) and in Drosophila (SONNENBLICK 1940). Of particular interest in the present case are the conclusions of CATCHESIDE and LEA (1945) in regard to the loss of the "largely inert" Y-chromosome of *Drosophila melanogaster* from the embryo following paternal irradiation; since the loss of the Y nearly always results in lethality, "The explanation presumably lies in the mechanical difficulties experienced by a dividing cell in which a dicentric bridge occurs as a result of sister union between chromatids at the breakage point." Such bridging, if it had occurred in the genetically inert heterochromatic set of male embryos of the mealy bug after low and moderate doses of paternal irradiation would probably have led to considerable lethality and obscured the demonstration of genetic inertness.

Under certain circumstances, coccid chromosomes are capable of forming bridges. In Steatococcus, bridges appear in spermatocytes but not in embryos after X-ray treatment (HUGHES-SCHRADER and RIS 1941). The bridges in the mealy bug embryos after high dosage paternal treatments have been difficult to analyze critically because the chromosomes have been severely fragmented; it is not known whether they were in any way the immediate result of the breakage; they may have been the product of chromosome stickiness induced by multiple fragmentation or formed by the irregular separation of small ring fragments (CHANDRA unpublished).

Evolutionary significance: The pseudococcids are a relatively unspecialized group at or near the start of an evolutionary series which extends to the armored scale insects (BALACHOWSKY 1942). Four chromosomal systems are now known for this series: the primitive XX-XO mechanism found so far only in the pseudococcid genus Puto (HUGHES-SCHRADER 1944, 1948); the lecanoid system (HUGHES-SCHRADER 1948; BROWN 1959); and, restricted to the armored scales, the diaspidid (BROWN and BENNETT 1957; BENNETT and BROWN 1958) and the Comstockiella systems (BROWN 1957). In the diaspidid system of the armored scales, the males become true haploids after the elimination of the paternal set at late cleavage. The paternal origin and primary inertness of the heterochromatic set of the mealy bugs thus make the lecanoid system appear to be a likely progenitor of true haplodiploid systems as originally suggested by SCHRADER and HUGHES-SCHRADER (1931), and more recently discussed by BROWN (1958). Evidence which is continuing to be accumulated indicates, however, that the Comstockiella system has been of major importance in the evolution of the armored scales (BROWN unpublished). In some species of armored scales, the Comstockiella and lecanoid systems may occur in different cysts of the same testis and are therefore indistinguishable until spermatogenesis; thus in certain respects the two systems would be expected to have the same evolutionary potentials.

SUMMARY

In the lecanoid chromosomal system, as exemplified by the mealy bug, *Planococcus citri* (Risso), one chromosome set becomes heterochromatic during embryogeny of the male and is maintained as such during development. At spermatogenesis, the first division is equational for both the euchromatic and heterochromatic chromosomes which are segregated from each other in the second; only the euchromatic derivatives form sperm. SCHRADER and HUGHES-SCHRADER suggested that the heterochromatic set is genetically inert and HUGHES-SCHRADER suggested that it is of paternal origin. Radiation studies were undertaken to test the SCHRADERS' hypotheses.

After paternal irradiation, the induced aberrations appear in the heterochromatic set of the male embryos while they occur in the euchromatic set after maternal treatment. HUGHES-SCHRADER's hypothesis of the paternal origin of the heterochromatic set is therefore confirmed.

After paternal irradiation of doses up to 30,000rep, dominant lethality is induced in daughters but not in sons. There is thus a clear picture of the failure of damage to the heterochromatic set to produce a detectable genetic effect and the hypothesis of the SCHRADERS in regard to its inertness is confirmed. These results do not rule out a secondary genetic or physiological function of the heterochromatic set. No breakage-fusion-bridge cycles, which would complicate the results whether the heterochromatic set were inert or not, were observed in the cytological studies after low-dosage treatments.

Further support for the hypothesis of inertness is provided by tests of X_1 males derived from fathers treated at 8,000 and 16,000r; these are as effective in fertilizing as their controls.

Because of the diffuse nature of the spindle attachment of coccid chromosomes, fragments can perpetuate themselves. Thus a complete break need not lead to a deficient chromosome. However, at subsequent oogenesis, the breakage products and other rearrangements would be expected to contribute to the formation of duplication-deficiency gametes. These expectations are borne out by the reduction in progeny, proportionate to original dosage, of X_1 females after either maternal or paternal treatment. In these progenies, the reduction in male offspring is proportionately greater than in the female; if the heterochromatic set is inert, the males would be expected to succumb to recessive as well as dominant lethals.

After maternal treatment, both sexes diminish with increasing dosage but the results are complicated by the effect of maternal age on the sequence in which the two sexes of offspring occur during oviposition (sexual dichronism) as well as on total progeny.

At high-dosage paternal treatments, 60, 90, and 120,000rep, the number of sons is drastically reduced while the number of daughters is about 40 percent of the controls. Cytological studies by CHANDRA (unpublished) indicate that the chromosomes are grossly damaged; chromosome bridging is prevalent and results in abnormal embryogenesis; the female survivors, triploid, diploid, and mosaic,

have undamaged chromosome sets which are therefore completely of maternal derivation.

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